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Genome Stability: A New Member of the RFC family

Dispatch

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Three distinct forms of replication factor C are known to play vital roles in genome replication and integrity in eukaryotic cells. A fourth such complex has recently been identified; initial results suggest that this new family member plays an important role during S phase.

Replication factor C (RFC) is a heteropentameric protein complex which plays essential roles in DNA replication and repair in eukaryotic cells [1]. In recent years, two RFC-like complexes (RLCs) have been identified, which have distinct functions in checkpoint signalling and the establishment of chromosome cohesion [2–6]. Each RLC is made up of the four small subunits of the archetypal RFC, but with the large subunit of that complex, Rfc1, replaced with an RFC-related protein, Rad24 or Ctf18. Now a fourth complex has been added to the family, with the discovery of a third yeast RLC, in which Elg1 replaces Rfc1 [7–9]. Cells lacking Elg1-RLC show chromosome instability and slow S-phase progression, suggesting a role for Elg1-RLC in genome replication. The presence of Elg1 proteins in other species hints at a conserved function for Elg1-RLC in all eukaryotic cells.

The primary function of RFC was first determined during studies of SV40 viral DNA replication [10], and is to load the 'sliding clamp' complex PCNA onto double-stranded DNA at primer–template junctions [11]. PCNA is a processivity factor for the DNA polymerases that replicate the bulk of the eukaryotic genome [11]. Because of its toroidal structure, PCNA must be catalytically opened and closed around the double-stranded DNA — this opening and closing is performed by the clamp loader RFC. Once loaded onto double-stranded DNA by RFC, PCNA acts to tether the polymerase, thereby conferring the necessary processivity on this enzyme complex.

In all eukaryotes, RFC is a complex of five related protein subunits (Figure 1): a large subunit, Rfc1, and four small subunits, Rfc2 to Rfc5. Each subunit is required for RFC function and for successful chromosome replication in yeast. All are members of the AAA$^+$ family of proteins [12] and contain seven well-conserved amino acid sequence motifs — called RFC boxes II through VIII — which are important for RFC complex formation and for PCNA loading [1].

In addition to interacting with Rfc1 in the archetypal RFC complex, the four small RFC subunits also form two distinct RLCs (Figure 1): Rad24-RLC and Ctf18-RLC [2-6]. Like RFC, Rad24-RLC is pentameric in structure. This complex functions in the DNA damage checkpoint in G1 and G2 phases of the cell cycle, and also in the intra-S phase checkpoint. In contrast, the Ctf18-RLC complex plays a vital role in chromosome cohesion, the process by which newly replicated sister chromatids remain physically associated until mitotic anaphase. Unlike RFC and Rad24-RLC, Ctf18-RLC has seven subunits — two additional proteins, Ctf8 and Dcc1, associate with the five subunit Ctf18-RLC core (Figure 1) [3–5].

What are the substrates of the Rad24-RLC and Ctf18-RLC complexes? Like RFC, Ctf18-RLC appears to load PCNA onto DNA [13,14]. In contrast to RFC and Ctf18-RLC, however, the Rad24-RLC complex has an altogether different substrate — a trimeric complex of the three checkpoint proteins Rad17, Mec3 and Ddc1 [15,16]. This complex — sometimes called the 9-1-1 complex after the fission yeast counterparts of Rad17, Mec3 and Ddc1 — forms a PCNA-like trimeric ring, and the RLC-Rad24 complex appears to play an identical role to RFC in opening and closing the 9-1-1 ring onto damaged DNA.

Now, three groups [7–9] working independently have identified a new RLC in yeast, Elg1-RLC. Their results provide intriguing new insights into the importance of RLC function for genome stability both in yeast and in higher eukaryotes. Two of the three groups [7,9] set out to identify mutations that increase the frequency of *Ty* element recombination. *Ty* elements are the largest family of naturally occurring repeated sequences in yeast. Although there are many *Ty* elements per genome, recombination between elements occurs at low frequency, suggesting the existence of a mechanism for suppressing *Ty* recombination. To investigate this, Kupiec and co-workers [9] isolated mutants that showed enhanced *Ty* recombination: one such mutation defined the *ELG1* gene. In a related study published recently in *Current Biology*, Durocher's group [7] analysed the frequency of gross chromosome rearrangements in yeast strains carrying mutations in nine poorly characterized *Ty* element regulators. One of the candidate genes elevated the gross chromosome rearrangement rate remarkably: that gene was *ELG1*.

In a third study, Brown and co-workers [8] took an altogether different approach, with the aim of identifying genes that play a part in stabilising compromised replication forks. They conducted genome-wide synthetic interaction screens with yeast strains carrying deletions of *MUS81* or *MMS4*. The Mus81 and Mms4 proteins are subunits of an endonuclease with a preference for branched DNA structures which is thought to be involved in the processing of stalled replication forks [17]. Approximately 4600 viable haploid deletion mutants were crossed with either *mus81Δ* or *mms4Δ* mutant strains, and double mutants showing lethality or extremely slow growth were identified. In this way, *ELG1* was identified for a third time: *elg1Δ mus81Δ* or *elg1Δ mms4Δ* double

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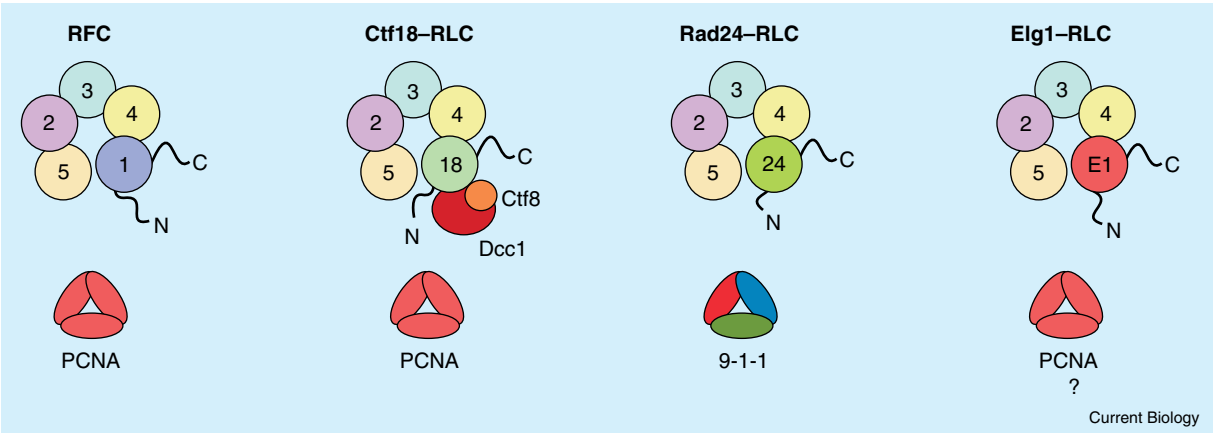

Figure 1. Schematic representation of RFC and the three RLCs in yeast with their cognate sliding clamps.

The complexes are defined by their large subunits, as the four small RFC subunits, designated 2–5, are common to all. Note that the large subunits possess extended amino- and carboxy-terminal regions of unknown function. Uniquely to date, the Ctf18-RLC has two additional non-RFC subunits Ctf8 and Dcc1. In addition, Rad24-RLC loads a distinct heterotrimeric sliding clamp, the 9-1-1 complex, comprising the budding yeast Rad17, Ddc1 and Mec3 proteins. As discussed in the text, initial results suggest that the Elg1-RLC most likely interacts with PCNA but confirmation of this awaits further analysis.

mutants are barely viable, while the parental strains grow well [8].

Subsequent sequence analysis revealed that the predicted Elg1 protein is related to the archetypal RFC subunits, as well as to Rad24 and Ctf18, and all three groups [7–9] were able to demonstrate that Elg1 physically associated with the small RFC subunits but not Rfc1, Rad24 or Ctf18, suggesting the existence of an altogether new RLC. Database searching also revealed the existence of Elg1 homologues in various other eukaryotic species, including humans, *Drosophila* and *Arabidopsis* [7–9], and the human Elg1 protein was shown to co-immunoprecipitate with human Rfc2 [7], suggesting that the structure of the complex is evolutionarily conserved. Whether the Elg1-RLC, like Ctf18-RLC, has additional subunits awaits purification of the complex.

What is the function of this new RLC? Although Elg1, like Rad24 and Ctf18, is non-essential, cells lacking Elg1-RLC display a variety of genome integrity defects. In addition to causing elevated *Ty* recombination, loss of Elg1 function in *elg1Δ* cells results in a slowing of progression though S phase and abnormal recovery from replication fork stalling induced by treatment with the DNA damaging agent MMS [7–9]. Consistent with a role for Elg1-RLC in the stabilisation or re-start of stalled replication forks, *elg1Δ* cells display synthetic genetic interactions with a range of genes with products that have been implicated in resolving collapsed replication fork structures, including *MUS81* and *MMS4* [8], as well as those involved in homologous recombination, such as *RAD52* [7–9].

In addition to a possible role in the stabilisation or re-start of stalled replication forks, genetic analysis suggests Elg1-RLC may play a role in lagging-strand DNA synthesis and/or Okazaki fragment processing during normal replication: *elg1Δ* cells display synthetic interactions with a number of genes with products required for these processes, such as the Fen1 and Dna2 nucleases or DNA ligase I [11]. Indeed, Durocher's group [7] report that Elg1 can be detected in association with Fen1 by immunoprecipitation. Elg1 also co-immunoprecipitates with the yeast PCNA protein Pol30, suggesting that Elg1-RLC interacts with this sliding clamp rather than with the 9-1-1 complex [7].

Taken together, these results indicate that Elg1 has an important role in the maintenance of genome stability, most likely in association with PCNA. If this is the case, how do the functions of RFC and the two PCNA-interacting RLCs, Ctf18-RLC and Elg1-RLC, differ from one another? Why are there three complexes, when all three appear to perform the same task, the loading of PCNA onto DNA? One possibility is that the RFC, Ctf18-RLC and Elg1-RLC preferentially recognise different DNA substrates. For Ctf18-RLC, it is believed that this enzyme complex performs its function at the specialised DNA structures that are sites of chromosome cohesion [3–5,14]. Presumably RFC and Elg1-RLC are unsuited to this task. Similarly, Elg1-RLC may recognise specific DNA structures that arise during replication fork stalling but not during unperturbed replication, the latter being the usual substrates for RFC.

A second possibility is that one or more of the RLC complexes functions, not as a PCNA loader, but as an unloader. It is estimated that the number of PCNA trimers in the cell is insufficient for lagging-strand DNA synthesis without active recycling being required. Elg1-RLC might be required for recycling PCNA, although as Elg1 is non-essential, this activity may be shared with RFC or other factors.

A third possibility is that RFC, Ctf18-RLC and Elg1-RLC recognise different forms of PCNA. This question comes into focus as a result of recent studies showing that yeast PCNA is present in several modified forms in the cell — mono-ubiquitinated, multi-ubiquitinated and SUMO-conjugated — with roles in postreplicative DNA repair [18,19]. Might the PCNA-specific RLC complexes load, or unload, one modified form in preference to the others? At present, there is no genetic or biochemical

evidence to support such an idea, but it seems unlikely that modifications of this type would have a neutral effect on the efficiency of clamp loading and unloading.

In conclusion, although we are still some distance from a complete understanding of the specialised roles of the RLCs, it is clear that they play important roles in a variety of processes central to genome maintenance and stability. Future studies, both biochemical and genetic, will no doubt shed further light on the function of these highly conserved clamp loading machines.

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